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Effect of bacosides, alcoholic extract of *Bacopa monniera* Linn. (brahmi), on experimental amnesia in mice

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To investigate the effect of bacosides (alcoholic extract of brahmi) on scopolamine (3 mg kg^{-1}, ip), sodium nitrite (75 mg kg^{-1}, ip) and BN52021 (15 mg kg^{-1}, ip) induced experimental amnesia in mice, using Morris water maze test, all the agents were administered 30 min before the acquisition trials on each day and repeated for 4 consecutive days, and on 5th day during the retrieval trials. Bacosides on anterograde administration (before training) in mice, significantly decreased the escape latency time (ELT) during the acquisition trials for 4 consecutive days and increased the time spent (TS) in target quadrant during the retrieval trials on 5th day, and on retrograde administration (after training) bacosides were found not to affect TS significantly. Bacosides also significantly decreased the ELT and increased the TS in mice treated anterogradely with scopolamine and sodium nitrite. Bacosides did not exhibit any significant effect on TS of mice treated retrogradely with sodium nitrite. On the other hand, bacosides significantly increased the TS of mice treated retrogradely with BN52021. On the basis of the present results it can be concluded that bacosides facilitate anterograde memory and attenuate anterograde experimental amnesia induced by scopolamine and sodium nitrite possibly by improving acetylcholine level and hypoxic conditions, respectively. Beside this bacosides also reversed BN52021 induced retrograde amnesia, probably due to increase in platelet activating factor (PAF) synthesis by enhancing cerebral glutamate level.

Keywords: Amnesia, Brahmi, Bacosides, Memory, Mice, Water maze.

Bacopa monniera (family: Scrophulariaceae) is a small creeper, commonly known as brahmi or jalanimba[1], distributed mainly in warm parts of the world. Phytochemical studies have shown that *B. monniera* contains many active constituents including alkaloids, saponins, bacosides A - F and nicotine; bacosides A and B being the major constituents[2-7]. The plant is used in the indigenous system of medicine for the treatment of cardiac, respiratory[8] and neuropharmacological disorders like insomnia, insanity, depression[9], anxiety[10,11], psychosis, epilepsy[12] and stress[13,14]. It also possesses, anti-inflammatory, analgesic[15], antipyretic[16], spasmolytic, antirheumatic, anticancer, antiulcer[17-19], astringent, bitter, cooling and anti-diarrhoeal properties[20]. An alcoholic extract of the plant was found to improve learning and memory in several test models[21-23] and to some extent corrected the abnormal behaviour[24]. *B. monniera* improves performance, information processing, learning and acquisition in animals and humans[22,25]. Brahmi prevents the rate of depletion of acetylcholine level in aged humans population[26,27] by inducing choline acetylase activity in the frontal cortex and hippocampus[28]. There are reports indicating that *B. monniera* increased superoxide dismutase, catalase, glutathione peroxidase[13], lipid peroxidase, glutamate, serotonin, protein kinase-c, protein activity and protein synthesis, especially in brain cells and decreased nor-epinephrine concentration in several brain regions like hippocampus, cerebral cortex, striatum and hypothalamus which play significant role in learning and memory processes[29]. In view of above reports, it is clear that *B. monniera* plays a appreciable role in various human disorders and improves learning (acquisition) and memory (retention) in animals and human beings significantly. Bacosides are believed to play a protective role in the synaptic functions of the

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nerves in the hippocampus, the seat of memory. Nerve impulses are transmitted across the synapses and their degeneration is believed to impair memory and cognition. But none of the studies gives clear idea about the neurotransmitter or retrograde messenger, which is particularly responsible for acquisition and retention of acquisition at cellular and molecular level. The present study has been designed to investigate the involvement of possible retrograde messenger in acquisition and retention of acquisition by alcoholic extract of brahmi in experimental amnesia, induced with scopolamine, sodium nitrite and BN52021, a platelet activating factor (PAF) receptor antagonist in mice, using water maze test[30].

Materials and Methods

Animals—Swiss albino mice (20-30 g) of either sex were procured from I.V.R.I., Izatnager, Bareilly. They were housed (12:12 hr. L:D cycle) in an animal house (at an ambient temperature of 20°-25°C; 50-55% RH) and had free access to water and standard diet (Kisan Feed India Ltd., Bombay.). All the animals used were naïve to water maze. The experiments were conducted in a semi-sound proof laboratory between 10:00 to 17:30 hrs. The research was conducted as per the guidelines of "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi.

Drugs and solutions—All the drug solutions were freshly prepared just prior to use. Alcoholic extract of brahmi or bacosides [Nivaran Herbal Pvt. Ltd. 8A, (basement) Dr. Ambedker Road, Chennai-24. India] was mixed with tween 80 and diluted with distilled water to get the final ratio of water and Tween 80 (9:1). BN52021 (Gift by Dr. P. Braquet, Institut Henri Beaufour, France) was dissolved in 0.5M dimethyl sulfoxide (DMSO). Scopolamine (Merck KgaA, 64271 Darmastadt, Germany) and sodium nitrite (s.d. fine chemicals Ltd. India) were dissolved in distilled water.

Apparatus—Escape latency time (ELT) and time spent (TS) of every animal was measured by employing the water maze test. The test allows the evaluation of spatial memory. Water provides a uniform intramaze environment, thus eliminating any olfactory interference. Food and water deprivation is not required in this test as required in other models. Water maze apparatus consists of a circular pool, made of a galvanized iron sheet having a diameter of 150 cm and height of 45 cm. The pool was filled with water up to a height of 30 cm. Water was made opaque with commercially available white color and maintained at 25°C. The pool was hypothetically divided into four equal quadrants with the help of two threads, fixed at right angle to each other, on the rim of the pool. A platform having an area of 11 cm^2 and height of 29 cm, was placed in the center of one of these four quadrants i.e. target quadrant. The platform was submerged 1 cm below the water surface.

Acquisition trials—Each mouse was placed in water maze apparatus for four consecutive days, with a 5 min interval between the trials from the midpoint of peripheral wall of each quadrant with its face towards the wall. The mice were allowed to swim for 2 min. After locating the hidden platform the mice were permitted to remain on it for 10 seconds before returning to the home cage. Mice that fail to locate the hidden platform within 2 min were placed on it by hand and scored as 2 min. The time taken by the mice to locate the hidden platform was noted down and was termed as escape latency time (ELT). Mean of the four-escape latency time was calculated for each day. This mean was used as index of acquisition or learning.

Retrieval trials—On 5^{th} day, platform was removed and time spent (TS) by the animal in each quadrant was noted down. The TS in target quadrant searching for missing platform is taken as an index of retrieval of memory.

Utmost care was taken not to change the relative location of water maze with respect to any object serving as a visual clue in the laboratory.

Experimental protocol—Fourteen groups of mice (n=5) were employed. All pharmacological agents [bacosides (30 mg kg^{-1}), sodium nitrite (75 mg kg^{-1}), scopolamine (3 mg kg^{-1}) and BN52021 (15 mg kg^{-1})] and their vehicles [normal saline (10 ml kg^{-1}), tween 80 (10%) (10 ml kg^{-1}), distilled water (10 ml kg^{-1}) and 0.5 M DMSO (10 ml kg^{-1}) were administered intraperitoneally (ip), 30 min before the first acquisition trial for 4 consecutive days i.e. day 1 to day 4 and 30 min before the first retrieval trial on 5^{th} day only. Bacosides (30 mg kg^{-1}, ip) were administered 5 min after the administration of scopolamine, sodium nitrite and BN52021 in mice, respectively.

Statistical analysis—All the results were statistically interpreted as mean ± SE. Data were analyzed using one-way analysis of variance

(ANOVA) followed by Dunnett's test. A value of $P<0.05$ was considered statistically significant.

Results

Effects of bacosides on learning and memory—In the control group, ELT was significantly decreased during consecutive learning trials on day 2, 3, 4 as compared to the means of day 1 (Fig. 1a). The time spent by the mice in the target quadrant (Q2) in search of missing platform was significantly higher as compared to time spent in the other quadrants Q1, Q3, Q4 during retrieval trials (Fig. 1b). Anterograde (before learning trials) administration of bacosides, significantly decreased the ELT during the learning trials for 4 consecutive days (Fig.1a) and increased the TS in target quadrant for searching the missing platform during the retrieval trial on 5th day. On the other hand, retrograde (after learning trials) administration of bacosides during the retrieval trials on 5th day only, did not produce any significant effect on TS in target quadrant for the search of missing platform, as compared to the control group (Fig. 1b).

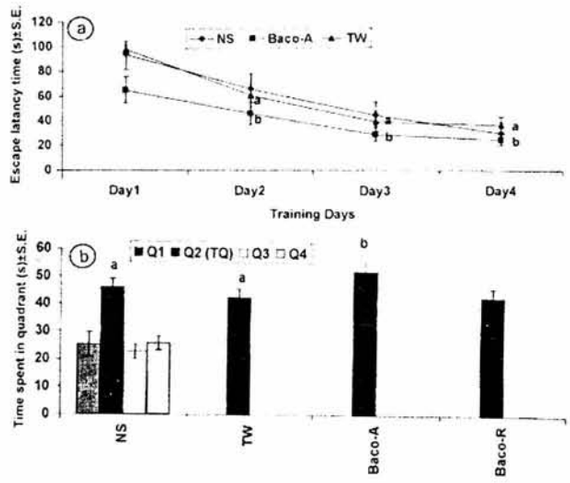

Fig. 1—(1a) -NS, Baco-A and TW represents administration of normal saline (10 ml kg^{-1}), tween 80 (10%) (10 ml kg^{-1}) and bacosides (30 mg kg^{-1}) intraperitoneally, 30 min before the first acquisition trial for 4 consecutive days. Each value represents mean±S.E. a= $p<0.05$ Vs ELT on day1 of tween 80 treated group. In bacosides treated group, b=$p<0.05$ Vs ELT of tween 80 treated group for the same day.

(1b) -NS, Baco-A and TW represents administration of normal saline (10 ml kg^{-1}), tween 80 (10%) (10 ml kg^{-1}) and bacosides (30 mg kg^{-1}) intraperitoneally, 30 min before the first acquisition trial for 4 consecutive days. Baco-R represents administration of bacosides (30 mg kg^{-1}, ip) 30 min before the first retrieval trial on 5th day only. Each value represents mean±S.E. a= $p<0.05$ Vs TS in other quadrants i.e. Q1 Q3 and Q4 of normal saline and tween 80 treated groups. In bacosides treated group, b=$p<0.05$ Vs TS in target quadrant (TQ) i.e. Q2 of tween 80 treated group.

Effect of scopolamine, sodium nitrite and BN52021 on learning and memory—Anterograde administration of scopolamine and sodium nitrite significantly attenuated the decrease in ELT during the learning trials for 4 consecutive days (Fig. 2a) and decreased the TS by the mice in target quadrant for searching the missing platform during the retrieval trials on 5th day (Fig. 2b) as compared to distilled water treated group. On the other hand, retrograde administration of scopolamine, did not produce any marked effect on time spent in target quadrant. But sodium nitrite significantly decreased the TS in target quadrant for the search of missing platform during the retrieval trials on 5th day (Fig. 2b). Anterograde administration

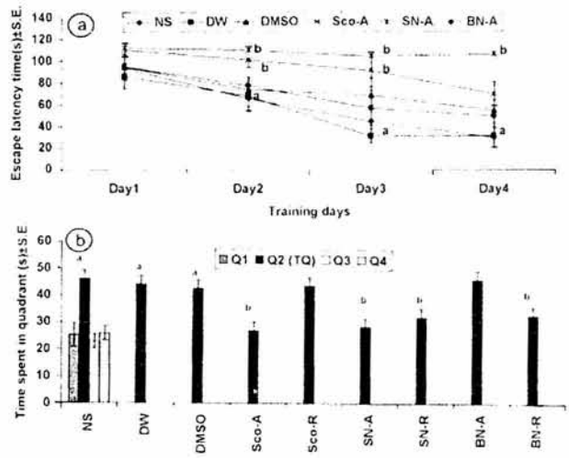

Fig. 2—(2a) -NS, DW, DMSO, Sco-A, SN-A and BN-A represents administration of normal saline (10 ml kg^{-1}), distilled water (10 ml kg^{-1}), dimethyl sulfoxide (0.5 M) (10 ml kg^{-1}), scopolamine (3 mg kg^{-1}), sodium nitrite (75 mg kg^{-1}) and BN52021 (15 mg kg^{-1}) intraperitoneally, 30 min before the first acquisition trial for 4 consecutive days. Each value represents mean±S.E. a= $p<0.05$ Vs ELT on day1 of distilled water treated group. In scopolamine and sodium nitrite treated group, b=$p<0.05$ Vs ELT of distilled water treated group for the same day.

(2b) -NS, DW, DMSO, Sco-A, SN-A and BN-A represents administration of normal saline (10 ml kg^{-1}), distilled water (10 ml kg^{-1}), dimethyl sulfoxide (0.5 M) (10 ml kg^{-1}), scopolamine (3mg kg^{-1}), sodium nitrite (75 mg kg^{-1}) and BN52021 (15mg kg^{-1}) intraperitoneally, 30 min before the first acquisition trial for 4 consecutive days. Sco-R, SN-R and BN-R represents administration of scopolamine (3 mg kg^{-1}), sodium nitrite (75 mg kg^{-1}) and BN52021 (15 mg kg^{-1}) intraperitoneally, 30 min before the first retrieval trial on 5th day only. Each value represents mean±S.E. a=$p<0.05$ Vs TS in other quadrants i.e. Q1 Q3 and Q4 of normal saline, tween 80 and dimethyl sulfoxide treated groups. In scopolamine, sodium nitrite and BN52021 treated groups, b=$p<0.05$ Vs TS in target quadrant (TQ) i.e. Q2 in distilled water and dimethyl sulfoxide treated groups, respectively.

of PAF antagonist BN52021 did not produce any significant effect on decrease in ELT during the learning trials for 4 consecutive days (Fig. 2a) and TS in target quadrant during the retrieval trials on 5th day (Fig. 2b) as compared to DMSO treated group. Moreover, retrograde administration of BN52021 significantly decreased the TS in target quadrant for searching of missing platform during the retrieval trials on 5th day (Fig. 2b).

Effect of bacosides on scopolamine induced amnesia—Anterograde administration of scopolamine was followed by bacosides, 30 min before the first learning trial for 4 consecutive days. Bacosides significantly reversed scopolamine induced attenuation of decrease in ELT during the learning trials (Fig. 3a) and also reversed the attenuation of higher TS in target quadrant by the mice to search the missing platform during the retrieval trials on 5th day (Fig. 3b).

Effect of bacosides on sodium nitrite induced amnesia—Anterograde administration of sodium nitrite was followed by bacosides, 30 min before the first learning trial for 4 consecutive days. Bacosides significantly overcome sodium nitrite induced attenuation of decrease in ELT during the learning trials (Fig. 3a). Bacosides also reversed the attenuation of sodium nitrite of higher TS in target quadrant to search the missing platform during the retrieval trials on 5th day (Fig. 3b). Retrograde administration of sodium nitrite was followed by bacosides, 30 min before the first retrieval trial on 5th day. Bacosides did not produce any significant effect on sodium nitrite induced decrease in TS in the target quadrant in search of missing platform during the retrieval trials on 5th day (Fig. 3b).

Effect of bacosides on BN52021 induced amnesia—Retrograde administration of BN52021 followed by bacosides, 30 min before the first retrieval trial on 5th day, significantly overcome, BN52021 induced attenuation of higher TS in target quadrant in search of missing platform during the retrieval trials on 5th day (Fig. 3b).

Discussion

Bacosides on anterograde administration (before training) in mice, significantly decreased the escape latency time (ELT) during the acquisition trials for 4 consecutive days and increased the time spent (TS) in target quadrant during the retrieval trials on 5th day. On retrograde administration (after training) bacosides did not show any significant effect on TS. This indicates that the bacosides improve acquisition when administered anterogradly in mice for 4 consecutive days but have no significant effect on retrieval of old memories. These findings support the learning and memory enhancing effect of *B. monniera* extract in animals and human beings as reported in various preclinical[24,28,29] and clinical[31-33] studies. Bacosides significantly decreased the ELT and increased the TS of mice treated anterogradly with scopolamine and sodium nitrite, indicating that bacosides attenuate scopolamine and sodium nitrite induced anterograde amnesia. These findings are further supported by the reports of Singh and Dhawan[29] showing that *B. monniera* improved

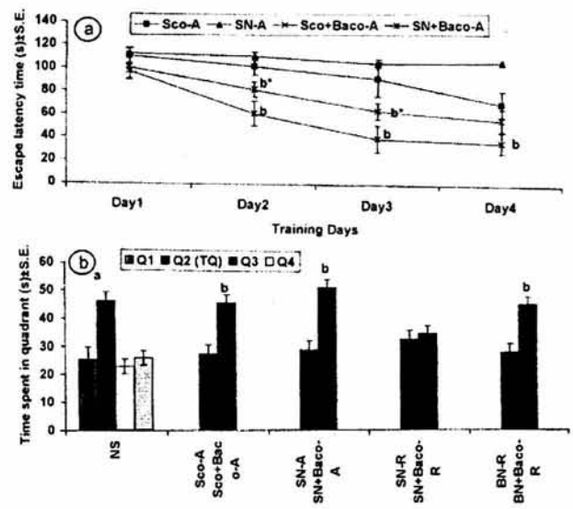

Fig. 3—(3a) -Sco-A, SN-A, Sco+Baco-A and SN+Baco-A represents administration of scopolamine (3 mg kg^{-1}), sodium nitrite (75 mg kg^{-1}), scopolamine (3 mg kg^{-1})+bacosides (30 mg kg^{-1}) and sodium nitrite (75 mg kg^{-1})+bacosides (30 mg kg^{-1}) intraperitoneally, 30 min before the first acquisition trial for 4 consecutive days. Each value represents mean±S.E. In sodium nitrite+bacosides and scopolamine+bacosides treated groups, b and b*= $p<0.05$ Vs ELT of sodium nitrite and scopolamine treated groups for the same day, respectively.

(3b) -NS, Sco-A, SN-A, Sco+Baco-A and SN+Baco-A represents administration of normal saline (10 ml kg^{-1}), scopolamine (3 mg kg^{-1}), sodium nitrite (75 mg kg^{-1}), scopolamine (3 mg kg^{-1})+bacosides (30 mg kg^{-1}) and sodium nitrite (75 mg kg^{-1})+bacosides (30 mg kg^{-1}) intraperitoneally, 30 min before the first acquisition trial for 4 consecutive days. SN-R, BN-R, SN+baco-R and BN+baco-R represents administration of sodium nitrite (75 mg kg^{-1}), BN52021 (15 mg kg^{-1}), sodium nitrite (75 mg kg^{-1})+bacosides (30 mg kg^{-1}) and BN52021 (15 mg kg^{-1})+ bacosides (30 mg kg^{-1}) intraperitoneally, 30 min before the first retrieval trial on 5th day only. Each value represents mean±S.E. b=$p<0.05$ Vs TS in target quadrant (TQ) i.e. Q2 of scopolamine, sodium nitrite and BN52021 treated groups, respectively.

scopolamine induced amnesia possibly due to its modulatory effect on the cholinergic system. Bhattacharya[34] reported that a herbal formulation with brahmi as a major constituent improves sodium nitrite induced hypoxia. Singh *et al.*[35] proposed that bacosides increase survival under hypoxic condition.

Bacosides significantly attenuated retrograde amnesia induced by platelet activating factor (PAF) receptor antagonist, BN52021. On the other hand bacosides does not have any significant effect on TS of mice treated retrogradly with sodium nitrite. On the basis of these results, it can be proposed that bacosides improve BN52021 induced retrograde amnesia possibly by enhancing the cerebral PAF level. This observation is further supported by the findings which indicate that PAF acts as a potent retrograde messenger in cognitive processes[36-38] and by the reports of Shukla *et al.*[39], indicating the potential of *B. monniera* in elevation of cerebral glutamate level that directly enhance PAF synthesis.

On the basis of these results it can be concluded that bacosides facilitate acquisition or learning. Bacosides reverse anterograde experimental amnesia of scopolamine and sodium nitrite possibly due to improving acetylcholine level and hypoxic conditions, respectively. Bacosides also reverse retrograde experimental amnesia of BN52021 possibly due to enhancement of PAF synthesis by elevating cerebral glutamate level at least in mice species.

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